Appendix 10 (Cont'd)

STUDY NUMBER: G89024 ( Study Completed )
DOSE GROUP/SEX: Group V ___ 96022-2 FEMALES

ANIMAL NUMBER: MACROSCOPIC & MICROSCOPIC FINDINGS

5108 (CONTINUED)

Microscopic Findings:

JEJUNUM : Autolysis

KIDNEY : Nephropathy, minimal
TUBULAR CELL ADENOMA (B)

LIVER : NEUROENDOCRINE CELL TUMOR (M), metastatic site. - routine sections and Lesions 3 and 4

LUNG : NEUROENDOCRINE CELL TUMOR (M), metastatic site

LYMPH NODE - MESENTERIC : NEUROENDOCRINE CELL TUMOR (M), metastatic site

MAMMARY GLAND : FIBROADENOMA (B). - Mass 2

OVARY : Cyst, NOS, bilateral
Hyperplasia, Sertoliform, mild

PANCREAS : NEUROENDOCRINE CELL TUMOR (M), metastatic site

PARATHYROID : Obs missing

PITUITARY : Cyst, NOS, pars distalis, multiple

STOMACH : Ectasia, glandular, fundus, moderate
Eosinophilic chief cells, moderate
Increased mucosal height, fundus, mild
NEUROENDOCRINE CELL TUMOR (M), Fundus, metastasizing

THYROID : FOLLICULAR CELL CARCINOMA (M)

THORACIC CAVITY : No microscopic evidence of macroscopic finding

(B)-BENIGN, (M)-MALIGNANT

....CONTINUED

Appendix 10 (Cont'd)

STUDY NUMBER: C89024 ( Study Completed )
DOSE GROUP/SEX: Group V — 96022-Z FEMALES

| ANIMAL NUMBER | MACROSCOPIC & MICROSCOPIC FINDINGS |
|---|---|
| 5108 | (CONTINUED) |

Microscopic Findings:

NO ABNORMALITIES DETECTED IN:
AORTA, BONE MARROW, BRAIN, COLON, ESOPHAGUS, ILEUM, PARATHYROID, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STOMACH, TRACHEA, URINARY BLADDER, UTERUS, LYMPH NODE - MANDIBULAR, SALIVARY GLAND - MANDIBULAR, SALIVARY GLAND - PAROTID, SALIVARY GLAND - SUBLINGUAL, STERNEBRA

/S/ 16 Dec 1992

Appendix 10 (Cont'd)

STUDY NUMBER: GS9024 ( Study Completed )
DOSE GROUP/SEX: Group V ___ P4023-I FINALES

ANIMAL NUMBER:MACROSCOPIC & MICROSCOPIC FINDINGS

$122 Mode of Death: Terminal Kill
Day of Death 737 Days

Macroscopic Findings:

ADRENAL : Both slightly enlarged

KIDNEY : Both pale
Cyst, cortical (2 mm diameter), cut surface, left kidney

MAMMARY GLAND : Mass 1: Firm, multilobular mass (4 X 3 X 3 cm), subcutis, right dorsum
Mass 2: Firm, nodular mass (10 X 9 X 9 cm), subcutis, left dorsum
Lesion 3: Firm nodule (1.5 X 1.5 X 1 cm), subcutis, right abdomen
Lesion 4: Soft nodule (2 X 1 X 0.5 cm), subcutis, left abdomen

STOMACH : Fundic mucosa appears thickened and folded
Raised, pale focus (3 mm diameter), mucosa, fundus

Microscopic Findings:

ADRENAL : Angiectasis, cortical, bilateral, multifocal
Cortical Hyperplasia, unilateral, multifocal, with cystic degeneration and vacuolation
Accessory adrenocortical tissue, capsular, bilateral, focal

HEART : Cardiomyopathy, moderate

KIDNEY : Nephropathy, severe
Mineralization, pelvic, unilateral, focal

LIVER : Eosinophilic cell focus, multiple, with angiectasis

MAMMARY GLAND : FIBROADENOMA [B], with ductal squamous cell hyperplasia, - Mass 1
FIBROADENOMA [B], - Mass 2
FIBROADENOMA [B], - Lesion 3

(B)=BENIGN. (M)=MALIGNANT

....CONTINUED

Appendix 10 (Cont'd)

STUDY NUMBER: GS9024 ( Study Completed )
DOSE GROUP/SEX: Group V ——→960322-E FEMALES
ANIMAL NUMBER:MACROSCOPIC & MICROSCOPIC FINDINGS

5122 (CONTINUED)

Microscopic Findings:

MAMMARY GLAND : FIBROADENOMA (B), - Lesion : FIBROADENOMA (B), adjacent to salivary glands

MESENTERY : Polyarteritis nodosa

OVARY : Hyperplasia, Sertoliform, mild

SPLEEN : Hematopoiesis, extramedullary, mild

STOMACH : Dilation, glandular, fundus
Ectasia, glandular, fundus, moderate
Eosinophilic chief cells, moderate
Increased mucosal height, fundus, moderate
NEUROENDOCRINE CELL TUMOR (B), fundus
ADENOMATOUS POLYP (B), fundus

THYROID : C-CELL ADENOMA (B)

NO ABNORMALITIES DETECTED IN:
AORTA, BONE MARROW, BRAIN, COLON, DUODENUM, ESOPHAGUS, EYE, ILEUM, JEJUNUM, LUNG, LYMPH NODE - MESENTERIC, PANCREAS, PARATHYROID, PITUITARY, SKELETAL MUSCLE, SKIN, SPINAL CORD, THYMUS, TRACHEA, URINARY BLADDER, UTERUS, LYMPH NODE - MANDIBULAR, SALIVARY GLAND - MANDIBULAR, SALIVARY GLAND - PAROTID, SALIVARY GLAND - SUBLINGUAL, STERNEBRA

/s/
12 Dec 1992

[B]=BENIGN, [M]=MALIGNANT

NDA 20,987

**PHARMACOLOGIST'S REVIEW OF NDA 20,987**
**(Amendments Dated May 12, 1999 and May 19, 1999)**

**Sponsor & Address:** Wyeth Ayerst Research
P.O. Box 8299
Philadelphia, PA 19101-82

**Reviewer:** Timothy W. Robison, Ph.D.
Pharmacologist, HFD-180

**Date of Submission:** May 12, 1999
May 19, 1999

**Date of HFD-180 Receipt:** May 13, 1999
May 20, 1999

**Date of Review:** June 7, 1999

**Drug:** Pantoprazole (PROTONIX™)

**Category**: Gastric parietal cell $H^+,K^+$-ATPase inhibitor; Proton Pump Inhibitor

**Submission Contents**:

The sponsor has responded to questions submitted by the Division in preparation for the [redacted]. Questions are listed below. For each question, the sponsor's response is summarized within quotations followed by an evaluation of each response.

1. Please explain the scientific rationale used for selection of doses and rat strain in the carcinogenicity study with pantoprazole in Fischer 344 rats? It appears that this study was initiated on July 20, 1992 and the In-Life phase should have been completed prior to September 7, 1994, [redacted] regarding pantoprazole was held. Could you please explain why the existence of this study or any preliminary findings were not communicated to the committee during the meeting on September 7, 1994?

**Sponsor's Response to First Question:** "In the Sprague-Dawley rat carcinogenicity study, body weights in the 200 mg/kg/day group were adversely affected, with mean weights decreased for male and female rats by 19% and 16%, respectively, compared with control weights. Survival was also poor for male rats at 200 mg/kg/day (4%). This data led to a decision to repeat the carcinogenicity study using lower doses. A high dose of 50 mg/kg/day was chosen based upon the Sprague-Dawley rat carcinogenicity study, in which this dose was associated with slight retardation of body weight gain.

The Fischer rat strain was chosen because of its reported better survival rate. From a pharmacokinetic standpoint, there were no differences between Sprague-Dawley and Fischer rats with regard to pantoprazole exposure."

**Evaluation:** No dose range finding studies were performed in order to select doses for Fischer 344 rat carcinogenicity study. The sponsor contended that the high dose of 200 mg/kg/day, used in the carcinogenicity study with pantoprazole in Sprague-Dawley rats, exceeded the maximum tolerated dose (MTD). Based upon these results with Sprague-Dawley rats, the sponsor selected doses of 5, 15, and 50 mg/ kg/day for the Fischer 344 rat carcinogenicity study. Dose selection for the carcinogenicity study with pantoprazole in Sprague-Dawley rats was judged to be adequate Survival rates were excellent up to 18-month of treatment (61-76% in the male vehicle-control and treatment groups and 74-86% in the female vehicle-control and treatment groups). Body weights up to 18 months in male treatment groups were 6 to 15% lower than the control, while body weights in female treatment groups at 50 and 200 mg/kg/day were 5% and 7% lower than control, respectively. There were low survival rates at termination for all groups including the vehicle-control (survival rates for the male vehicle-control and treatment groups ranged from 4 to 17%, while survival rates for the female vehicle-control and treatment groups ranged from 30-40%). Body weight loss during the last 6 month of the study for the male vehicle-control and treatment groups ranged from 16-27%. No monitoring for pathogens was conducted during the study. Hence, based upon excellent survival rates up to 18 months of treatment, the high dose of 200 mg/kg/day in the Sprague-Dawley rat carcinogenicity study was judged to have not exceeded the MTD. Furthermore, it is not scientifically valid to assess the MTD in one strain of rat and conduct carcinogenicity study in another strain of rat. In a 90-day dose range finding study with the thiol metabolite of pantoprazole (B8401-026) in Fischer rats, the sponsor included a group that received pantoprazole at 200 mg/kg/day as a comparator. For Fischer rats that received pantoprazole at 200 mg/kg/day, there was no mortality or effects on body weight gain. Further, observed histopathological findings were of a minor nature and would not be expected to have any impact on survival. The similar plasma AUC for the parent compound in Sprague-Dawley rats or Fischer rats that received pantoprazole at 50 mg/kg/day is not relevant as dose selection was based upon toxicity endpoints not exposure. Further, the toxicokinetics and toxicodynamics of pantoprazole had not been established in Fischer rats prior to the initiation of the carcinogenicity study in this rat strain. Thus, dose selection for the Fischer 344 rat carcinogenicity study was inadequate.

**Evaluation:** The question still remains of why did not the sponsor communicate the existence of the Fischer 344 rat carcinogenicity study or any preliminary findings to the Division . The sponsor does not appear to have been completely forthright in their disclosures to the committee regarding carcinogenicity studies with pantoprazole. Further, it is conceivable that information regarding dose selection for the Fischer rat carcinogenicity study as well as possibly other parameters such as observed effects, survival rates, and final body weights could have been communicated to the committee.

2. Incidences of granulocytic leukemia for male Fischer 344 rats at 0 (untreated control), 0 (vehicle control), 5, 15, and 50 mg/kg/day in Volume 1.062 on Page 23 were reported as 0/27, 0/16, 0/21, 1/20, and 2/27, respectively. However for the statistical analysis of this tumor, incidences for male rats at 0 (untreated control), 0 (vehicle control), 5, 15, and 50 mg/kg/day were reported as 0/50, 0/50, 0/50, 1/50, and 2/50, respectively. Was a reanalysis of the incidence of granulocytic leukemia performed in treatment groups? If so, please provide the following information regarding the reanalysis of samples: testing facility, study dates, GLP compliance, names of scientific personnel involved, and procedures used. Please indicate whether you have reported this information to the Agency previously.

**Sponsor's Response:** "The hematopoietic system was not a protocol tissue for the study and was listed as an organ in the computer to summarize hematopoietic neoplasms at any site and generalized reactive hyperplasia of lymphoid tissue. For nonprotocol tissues, the computer system that produced the Project Summary Table counted only animals with one or more findings in the given tissue as having been examined. Granulocytic leukemia is a multisystemic tumor and can occur in multiple organs when present. In support of this, the individual data for the 3 animals with this tumor demonstrate the multisystemic nature because 20 or more organs were infiltrated by granulocytic leukemia for each animal. In the original analysis, all animals were considered to have been examined histologically for this tumor. The original report included a statistical analysis for the tumor combination of all hematopoietic tumors, and the trend test for this combination was ( p value > 0.10). Combining these tumors is justified because morphology of cells in tissue sections is not adequate to differentiate granulocytic from large granular lymphocyte (LGL) leukemia (also referred to as mononuclear cell leukemia). For the Fischer rat carcinogenicity study with pantoprazole, incidences of this tumor in all groups including the controls (males 14-38%; females 2-14%) were similar to historical ranges for untreated animals on long term studies reported by Byk Gulden and in the literature."

**Evaluation:** The Project Summary Table, displaying tumor incidences in the hematopoietic system for the male control and treatment groups, on page 23 of Volume

1.062, was in error with regard to the total number of animals examined per group. This was reiterated by the sponsor [redacted] The total number examined should have been 50 rats/sex/group. The correct total number of animals examined per group and incidence of neoplastic findings for the hematopoietic system in male Fischer 344 rats that received pantoprazole are listed in the table below. The literature suggests that it is not appropriate to combine granulocytic leukemia with LGL leukemia. LGL leukemia is a clonal proliferation of lymphocytes. Granulocytic or myelogenous leukemia is a clonal malignant disease of hematopoietic tissue that is characterized by the proliferation of abnormal (leukemic) blast cells. Morphological classification of tumors of the hematopoietic system (In: Pathobiology of Tumours in Laboratory Animals. Editor: V.S. Turusov. International Agency for Research on Cancer, 1987.) is based upon (1) cell type and (2) spread. Clearly, LGL leukemia and granulocytic leukemia are derived from different cell types and should not be combined. Further, the sponsor has separately provided the incidences of granulocytic leukemia and LGL leukemia. Given the suggested difficulties in differentiating LGL leukemia and granulocytic leukemia, it might be expected that granulocytic leukemia would have been reported in other groups given that the incidence of LGL leukemia was high and relatively similar between groups; however, this was not the case. It should be noted, as described in greater detail under Question 3, that granulocytic leukemia is an extremely rare tumor for this rat strain. Data sheets for these male Fischer rats that received pantoprazole at 15 and 50 mg/kg/day for periods ≤104 weeks and diagnosed with granulocytic leukemia are attached in Appendix 1.

Neoplastic findings of the hematopoietic system in male Fischer 344 rats that received pantoprazole at 0 (untreated control), 0 (vehicle control), 5, 15, and 50 mg/kg/day for periods ≤ 104 weeks (n = 50/group).

| Hematopoietic System | 0 (UC) | 0 (VC) | 5 | 15 | 50 |
|---|---|---|---|---|---|
| leukemia, granulocytic | 0 | 0 | 0 | 1 (2%) | 2 (4%) |
| sarcoma, histiocytic | 1 | 0 | 0 | 0 | 0 |
| LGL leukemia | 19 | 13 | 16 | 7 | 13 |
| lymphoma/leukemia unclassified | 7 | 0 | 5 | 12 | 8 |
| reactive hyperplasia | 0 | 0 | 0 | 0 | 5 |

LGL leukemia = Large granular lymphocyte leukemia

3. Please provide the spontaneous tumor incidence for Fischer 344 rats in the testing facility over the period of 1990 to 1995.

**Sponsor's Response:** "Byk Gulden, which conducted the Fischer rat carcinogenicity study with pantoprazole, has conducted only one other carcinogenicity study with this rat strain from 1990 to 1995. There were no reported findings of granulocytic leukemia in this other study. For the reference, Spontaneous Neoplastic Lesions in the CDF (F-344)/[redacted] Rat [redacted] February 1990), the incidence of granulocytic (i.e., myelogenous) leukemia for male and female Fischer rats was 0.6% (5/846) and 0% (0/846), respectively. The range of incidence for granulocytic leukemia in male Fischer rats was 0 to 4%. For the reference, "Tumor Incidences in Fischer-344

Rats: NTP Historical Data" by J.K. Haseman, J. Arnold, and S.L. Eustis, In: Pathology of the Fischer Rat: Reference and Atlas. (Editors: Boorman GA, Eustis SL, Elwell MR et al.; Academic Press, 1990: Pages 555-564), the incidence of granulocytic leukemia was unknown as it appears that all leukemias were combined in this reference."

**Evaluation:** The testing laboratory at Byk Gulden has conducted only two studies in Fischer 344 rats and there were no findings of granulocytic leukemia in control animals of either study. The testing laboratory's lack of experience with Fischer 344 rats adds greater concern to the use of this rat strain in carcinogenicity studies with pantoprazole as described under Question 1. For the reference, Spontaneous Neoplastic Lesions in the CDF (F-344) — . Rat ——————— , February 1990), the range of incidence for granulocytic leukemia in male Fischer rats was 0 to 4%. It should be noted that all 5 findings of granulocytic leukemia were confined to 1 laboratory with a total of 126 rats. The upper incidence of 4% is based upon these 5 findings out of a total of 126 rats. There were no reported finding of granulocytic leukemia in male Fischer rats for the 9 other laboratories surveyed in this report (0/720). The reference "F344/N Rats: Tumor Incidence in Control Animals by Route and Vehicle of Administration prepared for the National Institute of Environmental Health Science (February 1998)" was consulted for incidence of granulocytic leukemia as shown in the table below. This data illustrates that granulocytic leukemia is a rare tumor for this rat strain. Incidence of granulocytic leukemia for male Fischer rats that received pantoprazole at 15 and 50 mg/kg/day for periods ≤104 weeks exceeds the spontaneous incidence of 0.09% by 22 and 44 times, respectively.

Incidence of granulocytic leukemia in male and female Fischer 344 rats [F344/N Rats: Tumor Incidence in Control Animals by Route and Vehicle of Administration prepared for the National Institute of Environmental Health Science (February 1998).

| Route/Vehicle | Male rats | Female rats |
|---|---|---|
| Dermal, Acetone | 0% (0/100) | 1% (1/100) |
| Dermal, Ethanol | 0.66% (2/302) | 0% (0/301) |
| Dermal, Neat | 0% (0/50) | 0% (0/51) |
| Gavage, corn oil | 0% (0/402) | 0% (0/401) |
| Gavage, water | 0% (0/50) | 0% (0/50) |
| Inhalation | 0% (0/50) | 0% (0/50) |
| Oral, Feed | 0% (0/904) | 0% (0/901) |
| Oral, Water | 0% (0/331) | 0% (0/330) |
| **Total** | **0.09% (2/2189)** | **0.045% (1/2184)** |

4. With reference to GTR-32977, ——————— displayed in Figure 4 (Volume 1.077, Page 35) suggest the presence of DNA adducts in liver DNA obtained from rats treated with pantoprazole at 200 mg/kg/day. For Figure 4, it appears that all samples were analyzed using the nuclease P1 enhancement procedure and separation in solvent system 1. For subsequent quantitation of DNA adducts as presented in Table 1 (Volume 1.077, Page 36), samples were assessed using solvent system 1, but without the nuclease P1 enhancement procedure. Why was the nuclease P1 enhancement procedure not used for quantitation of DNA adducts? Could the lack of use of the

nuclease P1 enhancement procedure be responsible for not confirming preliminary observations of DNA adducts obtained from rats treated with pantoprazole at 200 mg/kg/day?

**Sponsor's Response:** "In comparison with controls, the [redacted] illustrated in Figure 4c reveals one additional spot (labeled 3) in a DNA sample from a pantoprazole-treated rat prepared using nuclease P1 treatment. The same spot was present in the DNA samples from pantoprazole-treated rats used in the definitive experiments conducted to provide final evaluation under optimized conditions. Thus, the preliminary results of an additional spot observed with DNA from pantoprazole-treated rats were confirmed in the final evaluation. In the final evaluation experiments, this extra spot, however, did not exhibit a statistically significant difference in putative adducts (i.e., radioactivity) compared with the appropriate area from control samples. This spot may represent an indigenous modification of DNA, like the major spot 1 seen in all samples, with detection facilitated by the 4-week pantoprazole exposure. Such facilitation may relate to the fact that radioactivity in the nearby major spot 1 is diminished in pantoprazole samples. It even disappeared altogether in the lansoprazole sample (Figure 4e)."

1 PAGE(S) REDACTED

**Table 1. Number of adducts/$10^9$ nucleotides detected in the Omeprazole and Pantoprazole spots and background values for the equivalent areas in controls**

| | | Exp. 1 | Exp. 2 | Exp. 3 | Mean | SD | Average | SD | Significance |
|---|---|---|---|---|---|---|---|---|---|
| Omeprazole | | | | | | | | | |
| 200mg/kg | 1 | | | | 2.28 | 1.5 | | | |
| | 2 | | | | 2.11 | 0.6 | 2.30 | 0.2 | NS |
| | 3 | | | | 2.5 | 0.9 | | | |
| 600mg/kg | 1 | | | | 2.82 | 0.6 | | | |
| | 2 | | | | 2.81 | 0.5 | 2.52 | 0.5 | NS |
| | 3 | | | | 1.93 | 0.8 | | | |
| Control | 1 | | | | 2.13 | 1.8 | | | |
| (Background) | 2 | | | | 2.18 | 2.3 | 1.90 | 0.5 | |
| | 3 | | | | 1.29 | 0.9 | | | |
| Pantoprazole | | | | | | | | | |
| 200mg/kg | 1 | | | | 8.04 | 5.8 | | | |
| | 2 | | | | 11.74 | 6.0 | 10.26 | 1.96 | NS |
| | 3 | | | | 11 | 3.7 | | | |
| Control | 1 | | | | 7.22 | 4.2 | | | |
| (Background) | 2 | | | | 9.11 | 5.8 | 7.53 | 1.5 | |
| | 3 | | | | 6.27 | 3.2 | | | |

* No visible spot
NS, no significant difference between the dosed and the corresponding control (background) samples.

For data presented in Table 1, samples were separated using solvent system 1 without the use of a DNA adduct enhancement procedure.

**Evaluation:** [redacted] presented in Figure 4 (see above) suggested the presence of a DNA adduct(s) in liver DNA obtained from rats treated with pantoprazole. A distinct adduct spot (denoted as #3) for pantoprazole samples was located to the lower left of the main adduct spot that was seen in both control and treated samples. This distinct adduct spot suggests that pantoprazole or one of its metabolites directly interacts with DNA. For [redacted] presented in Figure 4, all samples were prepared using the nuclease P1 enhancement procedure prior to $^{32}$P-labeling and separation using solvent system 1. The sponsor in definitive experiments did not use the nuclease P1 enhancement procedure prior to $^{32}$P-labeling and separation using solvent system 1. Quantitation of DNA adducts under these conditions is presented in Table 1 (see above). Table 1 apparently considers only the quantity for one DNA adduct spot and not the total quantity of DNA adducts. Based upon the data in Table 1, the sponsor did not consider pantoprazole to be positive in the $^{32}$P-Postlabeling assay. For results presented in Table 1, the lack of use of nuclease P1 enhancement in these experiments may have obscured results and dampened a potential positive response for pantoprazole. Nuclease P1 is used to cleave deoxyribonucleoside 3`-monphosphates of normal nucleotides to deoxyribonucleosides, which do not serve as substrates for $^{32}$P-labeling mediated by polynucleotide kinase, while most adducted nucleotides are, in general, totally or partially resistant to the 3'-desphosphorylating action of nuclease P1 (Carcinogenesis 7: 1543-1551, 1986). Thus, use of nuclease P1 enhances sensitivity for detection of DNA adducts and in general removes normal

nucleotides, which would otherwise increase the background. Further, the rate of enzymatic $^{32}P$-labeling of nucleotide-adducts is, in general, slower than observed for normal nucleotides (Chem. Res. Toxicol. 12: 68-77, 1999; Carcinogenesis 9: 1687-1693, 1988; Mutagenesis 8: 121-126, 1993; Carcinogenesis 14: 2463-2469, 1993). Without the use of a nucleotide-DNA adduct enrichment procedure, such as the nuclease P1 procedure, it is conceivable that the $^{32}P$-ATP labeling substrate in the assay could be depleted by the labeling of normal nucleotide prior to any labeling of nucleotide-adducts. The quantitation of nucleotide-adducts by the sponsor is considered to be unreliable. The Genetic Toxicology Committee concluded that there was reasonable qualitative evidence for the formation of DNA adducts from pantoprazole; however, without further information, nothing can be stated with regard to absolute levels of adducts

5. For the amendment submitted April 26, 1999 in reference to GTR-32977, analysis of [redacted] that were prepared using chromatography system 1 without DNA adduct enhancement for preparation of data in Table 1 were difficult or impossible to interpret due to either possible [redacted] or difficulties in clearly photocopying [redacted] Resolution of adducts appeared to be poor based upon both photocopies [redacted] and hand-drawn diagrams of [redacted] Please explain how spots that appear to be poorly resolved from one another were subdivided into individual adducts. Please explain how consistency was maintained in the subdivision of these spots, that appear to be poorly resolved, into individual adducts. What is the reliability of data presented in Table 1 (Volume 1.077, Page 36)?

Sponsor's Response: "In the method used, [redacted] The discrimination of individual spots is done visually. The tracing thus obtained represents all of the spots on a single plate from a single experiment. For quantitation, the spots were always normalized to the area of the right-hand side of the plate selected for background (labeled Bg).

Evaluation: The question still remains as resolution of individual nucleotide-adduct spots appeared poor and it was difficult to determine large masses were subdivided into individual spots. Further, it is difficult to determine how consistency was maintained in the subdivision of large masses into individual nucleotide-adduct spots from sample to sample. Representative hand-drawn diagrams of [redacted] are attached in Appendix 3. As noted under Question 4, quantitation of nucleotide-adducts was considered to be unreliable and concurrence regarding this opinion was obtained from the Genetic Toxicology Committee

6. With regard the Syrian hamster embryo (SHE) cell assays performed with pantoprazole and its thiol metabolite (B8401-026), could the sponsor elaborate on their

efforts to control potential sources of variability in the assay as follows: (1) screening of SHE cell isolates to find a useful one; (2) screening lots of fetal bovine serum to ensure that they support SHE cell growth and transformation; and (3) level of induction of morphological transformation produced by positive controls. It has recently been reported that performance of the assay at pH 6.70 increases the transformation response up to 10-fold as compared to that observed at pH 7.10-7.35 (Kerckaert et al. Mutation Research 356: 65-84, 1996; LeBoeuf et al. Mutation Research 356: 85-127, 1996). Could you please assess the impact of these potential sources of variation as well as performance of the assay at pH 7.2 rather than pH 6.7 on the negative outcome of assays with pantoprazole and B8401-026.

**Sponsor's Response**: "The SHE transformation assays with pantoprazole and its thiol metabolite were conducted in 1992, which was 4 years before the modified procedures were reported by Kerckaert et al. Mutation Research 356: 65-84, 1996 and ; LeBoeuf et al. Mutation Research 356: 85-127, 1996. Any assessment of potential differences in outcome would be highly speculative. Large stocks of SHE cells were cryopreserved and screened for susceptibility to transformation by benzo[a]pyrene in the presence of metabolic activation and N-methyl-N'-nitro-N-nitroso-guanidine in the absence of metabolic activation for selection of responsive batches. SHE cell growth and transformation was adequately demonstrated by response to the positive controls. This was ensured by using irradiated feeder cells which precluded the need for screening the fetal calf serum. The levels of induced transformation by positive controls were generally consistent and appeared to be in agreement with published literature."

**Evaluation**: The sponsor's response appears to be adequate.

7. Could the sponsor please explain the sporadic occurrence of hepatocellular adenoma, hepatocellular carcinoma, thyroid C-cell adenoma, and malignant neuroendocrine cell tumor observed in Sprague-Dawley rats treated with pantoprazole in the 6-month and 12-month toxicology studies?

**Sponsor's Response**: "Liver tumors were not considered to be related to pantoprazole administration based upon on their sporadic occurrence in treated animals, the spontaneous occurrence in a control, and the absence of a dose-related response. Incidences of hepatocellular carcinoma were within the historical control (0-5.3%) reported by ——————— for male Sprague-Dawley rats in 12-month studies. Therefore, the liver tumors observed in the 6- and 12-month studies are not considered to be related to pantoprazole exposure. In these studies, thyroid C-cell adenoma was not considered pantoprazole related because of the sporadic occurrence of the tumors, the spontaneous occurrence in a control, and the absence of a dose-related response. C-cell adenomas occur spontaneously and increase in incidence with age. Furthermore, during the treatment phase of the 6-month study, the incidence of C-cell adenoma was within the historical control range (3.6-7.6%) reported by ——— ——————— for male Sprague-Dawley rats in 12-month studies. In the recovery

phase of the pantoprazole 12-month study, the incidence of C-cell adenoma was comparable between the control and treated groups. Therefore, the thyroid tumors observed in the 6- and 12-month studies are not considered to be related to pantoprazole treatment."

Evaluation: In the two-year carcinogenicity study with pantoprazole in Sprague-Dawley rats, there were findings of dose-related increased incidences of hepatocellular adenomas and carcinomas in the liver at doses of 0.5, 5, 50, and 200 mg/kg/day and an increased incidence of follicular cell adenomas and carcinomas in the thyroid gland at the high dose of 200 mg/kg/day. In the 6-month chronic toxicology study with Sprague-Dawley rats, there was a finding of a hepatocellular adenoma in 1 male rat at the high dose of 320 mg/kg/day. This finding may reinforce the findings of the two-year carcinogenicity study. There were no similar findings in the concurrent control group. It is not appropriate to apply the historical control range reported by ——— for Sprague-Dawley rats in 12-month studies, given that the study in question was a 6-month study. Further, the data base that was used by ——— to construct the historical control ranges of neoplastic findings for Sprague-Dawley rats in 12-month studies is extremely small and its reliability is unknown. In the 6-month toxicology study, a thyroid C-cell adenoma was observed for 1 female rat at 16 mg/kg/day. There were no similar findings in the concurrent control group. Again, it is not appropriate to apply the historical control range reported by ——— for Sprague-Dawley rats in 12-month studies, given that the study in question was a 6-month study. For the 12-month chronic toxicology in Sprague Dawley rats at the low dose of 5 mg/kg/day, there were findings of a hepatocellular adenoma in 1 male rat and a hepatocellular carcinoma in another male rat. These findings may potentially reinforce the findings of two-year carcinogenicity study. Although, there was a lack of dose response relationship for these findings, there were no similar findings in the concurrent control group. Again, the data base that was used by ——— to construct the historical control ranges of neoplastic findings for Sprague-Dawley rats in 12-month studies is extremely small and its reliability is unknown. The finding of a hepatocellular carcinoma in a control male at the end of 9-month free recovery period following the 12-month treatment period (i.e., day 639 or a total of 21 months on study) would appear to have no relevance given the differences in ages of the animals. Further, histopathological reports for animals that survived to the end of 9-month drug-free recovery period following the 12-month treatment period (i.e., day 639) were based upon incomplete analyses of all tissues and organs as examinations were confined to the stomach and macroscopic abnormalities (see Page 18 of Volume 1.031), and should not be used as a reference. At the end of the 9-month free recovery period following the 12-month treatment period, there was a finding of a malignant neuroendocrine cell tumor for 1 female rat in the 5 mg/kg/day group. There were no tumor findings in the stomach at the end of the 12-month treatment period. This finding would suggest that tumorigenic processes initiated during the treatment period persisted through the recovery period to result in the production of a tumor.

8. Please provide direct comparisons of 8-week old [redacted] Sprague-Dawley rats (used in the carcinogenicity study conducted at [redacted] 1989-1993) and 6-week old Fischer CDF/F-344 rats (used in the carcinogenicity study conducted at Byk Gulden, Hamburg, Germany, 1992-1996) with respect to pharmacokinetics, toxicokinetics, pharmacodynamics, and toxicological responses found with pantoprazole treatment.

Sponsor's Response: "A comparative study was conducted with 12-month old Sprague-Dawley, Wistar, and Fischer rats that received pantoprazole by oral gavage at doses of 0, 0.8, or 4 mg/kg/day for 3 months. Serum gastrin levels were elevated at 4 mg/kg/day in all strains. Morphological alterations in the mucosa of the gastric fundus were found in pantoprazole-treated animals of all three rat strains. Sections of the gastric fundus showed a dose-related increase in Grimelius-positive cells in the 4 mg/kg/day group of all three strains compared with controls. The results of this study illustrate a comparable pharmacodynamic effect of pantoprazole in the three rat strains. Administration of pantoprazole to male Fischer rats at a dose of 50 mg/kg/day by oral gavage produced plasma AUC values for the parent compound of 35.2 µg*hr/mL at 1 year and 32.4 µg*hr/mL at 2 years. Administration of pantoprazole at a dose of 50 mg/kg/day by oral gavage to male and female Sprague-Dawley rats produced plasma AUC values for the parent compound of 47.5 µg*hr/mL on day 7 and 42.6 µg*hr/mL on day 30. From a pharmacokinetic point of view, it can be concluded that Fischer rats behave like Sprague-Dawley rats as both are comparably exposed to the compound at the same dose level over extended periods of time.

Evaluation: The sponsor has not addressed the specific question. The study with aged rats of three different strains involves the use of sub-therapeutic doses of pantoprazole and cannot be considered a toxicology study. Further, the dose range is too narrow to detect differences between the three rat strains. The question seeks to understand if the toxicokinetics and toxicodynamics of pantoprazole had been thoroughly defined in Fischer rats prior to the initiation of the carcinogenicity study with this rat strain and how do they compare with toxicokinetics and toxicodynamics of pantoprazole in Sprague-Dawley rats, which have been extensively characterized. The metabolism of pantoprazole in Fischer rats has not been determined so a simple comparison of AUC values for the parent compound between these two rat strains has little value. The comparison of plasma AUC values for the parent compound following subacute treatment of Sprague-Dawley rats as compared to chronic treatment of Fischer rats can be potentially misleading given that pantoprazole is a weak inducer of cytochrome P450 activities as well as UDP-glucuronyl transferase activity. A dose range finding study in Fischer rats to define toxicity endpoints was not conducted prior to initiation of the carcinogenicity study in this rat strain (i.e., the toxicokinetics and toxicodynamics of pantoprazole in Fischer rats were unknown prior to initiation of the carcinogenicity study). Relative similarities in plasma AUC values for parent compound between Fischer rats and Sprague-Dawley rats have no relationship to the use of toxicity endpoints for selection of appropriate doses for a carcinogenicity study given the genotoxic findings with pantoprazole.

## SUMMARY AND EVALUATION

Pantoprazole is an inhibitor of gastric parietal cell $H^+,K^+$-ATPase under development for treatment of GERD. In support of the development of this agent, a carcinogenicity study with pantoprazole in Fischer 344 rats at doses of 5, 15, and 50 mg/kg/day was conducted by Byk Gulden of Konstanz, Germany. In this study, there were findings of granulocytic leukemia in the male mid and high dose groups. Granulocytic leukemia is a rare tumor for this rat strain. With regard to the etiology and pathogenesis of granulocytic leukemia, three well-documented environmental factors are established causal agents: (1) high dose radiation, (2) chronic benzene exposure, and (3) alkylating agents (Lichtman, MA. Acute myelogenous leukemia and Chronic myelogenous leukemia and related disorders. In: Williams Hematology, 5th Edition. Editors: Beutler E, Lichtman MA, Coller BS, and Kipps TJ. McGraw-Hill, Inc. Chapter 27, Pages 272-298 and Chapter 29, Pages 298-324, 1995). Pantoprazole has been demonstrated to be a genotoxic agent that possesses both clastogenic and mutagenic activities. The findings of granulocytic leukemia in male rats in the mid and high dose treatment groups should be confirmed by an independent pathologist.

Recommendations: The sponsor should be asked to provide information as described below.

1. With regard to the 104-week carcinogenicity study with pantoprazole in Fischer 344 rats, for male number 83 at 15 mg/kg/day and male numbers 41 and 410 at 50 mg/kg/day that were diagnosed with granulocytic leukemia, the sponsor should be requested to provide all organ and tissue histopathology slides for independent verification of this finding.

2. With regard to the amendment dated April 26, 1999 that contained individual sample [redacted] and hand-drawn diagrams, could sponsor specifically identify which samples were used in the construction of Figure 4 (Volume 1.077 of the NDA, Page 35).

/S/
Timothy W. Robison, Ph.D. — Date 6-7-99

/S/ 6/10/99

Appendix 1: Data sheets for animals in the Fischer rat carcinogenicity study diagnosed with granulocytic leukemia.

Appendix 2: [redacted]

Appendix 3: Hand-drawn diagrams [redacted] ($^{32}$P-Postlabeling experiments).

cc:
Orig NDA 20,987
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Robison

**Appendix 1:** Data sheets for animals in the Fischer rat carcinogenicity study diagnosed with granulocytic leukemia.

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**Appendix 3:** Hand-drawn diagrams of [redacted] $^{32}$P-Postlabeling experiments).

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Walsh

NDA 20,987

**PHARMACOLOGIST'S REVIEW OF NDA 20,987**
**(Amendments Dated April 26, 1999 and May 11, 1999)**

**Sponsor & Address:** Wyeth Ayerst Research
P.O. Box 8299
Philadelphia, PA 19101-82

MAY 21 1999

**Reviewer:** Timothy W. Robison, Ph.D.
Pharmacologist, HFD-180

**Date of Submission:** April 26, 1999
May 11, 1999

**Date of HFD-180 Receipt:** April 27, 1999
May 12, 1999

**Date of Review:** May 21, 1999

**Drug:** Pantoprazole (PROTONIX™)

**Category:** Gastric parietal cell $H^+,K^+$-ATPase inhibitor; Proton Pump Inhibitor.

**Submission Contents:**

In the present submissions, the sponsor has responded to a request from the Division dated April 13, 1999 regarding $^{32}$P-Postlabeling Studies presented in General Technical Report (GTR) #32977 (in NDA Volume 1.077). For each point, a summary of the sponsor response is presented within quotations followed by an evaluation of this response.

1. "In the Introduction on page 2 of the current submission, the sponsor has claimed that exposure to pantoprazole is much greater in rodents than that achieved with comparable dosages of other proton pump inhibitors. They further stated that systemic exposure for omeprazole at 600 mg/kg/day and lansoprazole at 1200 mg/kg/day were similar to pantoprazole at 200 mg/kg/day."

**Evaluation:** In a 6-month dose range finding study with pantoprazole in Sprague-Dawley rats, 200 mg/kg/day was identified as the maximum tolerated dose. Doses of omeprazole at 200 and 600 mg/kg and lansoprazole at 200 and 1200 mg/kg used in these studies were unusually high and exceeded doses used in respective carcinogenicity studies with Sprague-Dawley rats (i.e., > maximum tolerated doses). Furtner, based upon toxicity endpoints as described below for omeprazole and lansoprazole, pantoprazole at 200 mg/kg/day cannot be equated to omeprazole at 600 mg/kg/day or lansoprazole at 1200 mg/kg/day.

For a 6-month toxicology study with omeprazole in Sprague Dawley rats submitted under NDA 19,810 (Astra Laboratories, Inc.), a dose of 138 mg/kg/day produced significant histopathological findings in the bone marrow, lungs, and liver. Bone marrow hyperplasia was observed for 8% of male rats. Findings for the lung consisted of an increased incidence of peribronchiolar lymphoid hyperplasia with or without lymphocyte infiltration. Findings in the liver consisted of an increased incidence of periportal leukocyte infiltration with and without microfocal necrosis.

For a 3-month dose range finding study with lansoprazole in Sprague Dawley rats included in NDA 20,406 (TAP Pharmaceuticals, Inc. Deerfield, IL), a dose of 300 and 600 mg/kg/day depressed body weight gains to 26-35 and 59-63% of the control, respectively. Histopathological changes at 300 and 600 mg/kg/day were found in the testes, bone marrow, spleen, thymus, and kidney. For the testes, findings included epididymal hypospermia, aspermia, and bilateral, multifocal testicular atrophy. For the bone marrow, hypocellularity was observed. For the spleen, the incidence of capsular inflammation was increased. For the thymus, the incidence of atrophy was increased.

Systemic exposure to the parent compound for pantoprazole at 200 mg/kg/day and omeprazole at 600 mg/kg/day were roughly equivalent; however, the sponsor has completely ignored metabolite levels. No data was provided for lansoprazole at 1200 mg/kg/day. **Systemic exposure is defined by exposures (i.e., AUCs) to the parent compound + metabolites.** Metabolite levels have not been considered by the sponsor for either of the three compounds. First pass hepatic metabolism of omeprazole and lansoprazole appears to be more extensive than observed for pantoprazole. Metabolite levels observed with omeprazole and lansoprazole are most likely greater than those observed with pantoprazole.

2. "The sponsor provided details of methodology used for preparation of $^{32}$P-labeled adducts, chromatographic separation of adducts, and quantitation of adducts."

Evaluation: ———— in preliminary studies (see Figure 4 below) suggested the presence of a distinct DNA adduct spot with pantoprazole samples prepared from livers of rats treated with pantoprazole at 200 mg/kg/day for 4 weeks. A faint DNA adduct spot was evident for omeprazole samples prepared from livers of rats treated with omeprazole at 600 mg/kg/day for 4 weeks. It should be noted that omeprazole at 200 mg/kg/day and lansoprazole at 200 and 1200 mg/kg/day were apparently negative.

The omeprazole dose at 600 mg/kg/day was unusually high and exceeded the highest dose used in the Sprague Dawley rat carcinogenicity study by 4-fold. Samples shown in _____ presented in Figure 4 were prepared using a nuclease P1 enhancement procedure prior to enzymatic $^{32}$P-labeling and separation using solvent system 1. The sponsor subsequently reassessed samples by using solvent system 1, but without the use of a DNA adduct enhancement procedure prior to enzymatic $^{32}$P-labeling. Data for reassessed samples is presented in Table 1 (see below). This table suggests no quantitative differences between the pantoprazole adduct spot and an equivalent control area. For results presented in Table 1, the lack of use of the nuclease P1 enhancement procedure in these experiments may have obscured results and dampened a potential positive response for pantoprazole. Clearly, preliminary results indicated a distinct DNA adduct spot for pantoprazole samples. Nuclease P1 is used to cleave deoxyribonucleoside 3`-monphosphates of normal nucleotides to deoxyribonucleosides, which do not serve as substrates for $^{32}$P-labeling mediated by polynucleotide kinase, while most adducted nucleotides are, in general, totally or partially resistant to the 3'-desphosphorylating action of nuclease P1. Thus, use of nuclease P1 enhances sensitivity for detection of DNA adducts and removes normal nucleotides, which would otherwise increase the background. Further, enzymatic labeling efficiency of nucleotide-adducts can vary significantly from that observed with normal nucleotides (Mutagenesis 8: 121-126, 1993; Carcinogenesis 18:2367-2371, 1997; Chemical Research in Toxicology 12: 68-77, 1999; and Chemical Research in Toxicology 12: 93-99, 1999). An adduct enrichment procedure, such as the nuclease P1 enhancement procedure, may be essential to labeling adducts due to difference in labeling efficiency. Potentially, all $^{32}$P-ATP available in the reaction could be consumed by labeling normal nucleotides before any nucleotide-adducts are labeled in the absence of an adduct enrichment procedure. Please see comments regarding resolution of adducts with solvent system 1 below.

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**Table 1. Number of adducts/$10^8$ nucleotides detected in the Omeprazole and Pantoprazole spots and background values for the equivalent areas in controls**

| | | Exp. 1 | Exp. 2 | Exp. 3 | Mean | SD | Average | SD | Significance |
|---|---|---|---|---|---|---|---|---|---|
| Omeprazole 200mg/kg | 1 | | | | 2.28 | 1.5 | | | |
| | 2 | | | | 2.11 | 0.6 | 2.30 | 0.2 | NS |
| | 3 | | | | 2.5 | 0.9 | | | |
| 600mg/kg | 1 | | | | 2.82 | 0.6 | | | |
| | 2 | | | | 2.81 | 0.5 | 2.52 | 0.5 | NS |
| | 3 | | | | 1.93 | 0.8 | | | |
| Control (Background) | 1 | | | | 2.13 | 1.8 | | | |
| | 2 | | | | 2.18 | 2.3 | 1.90 | 0.5 | |
| | 3 | | | | 1.29 | 0.9 | | | |
| Pantoprazole 200mg/kg | 1 | | | | 8.04 | 5.8 | | | |
| | 2 | | | | 11.74 | 6.0 | 10.26 | 1.96 | NS |
| | 3 | | | | 11 | 3.7 | | | |
| Control (Background) | 1 | | | | 7.22 | 4.2 | | | |
| | 2 | | | | 9.11 | 5.8 | 7.53 | 1.5 | |
| | 3 | | | | 6.27 | 3.2 | | | |

* No visible spot
NS, no significant difference between the dosed and the corresponding control (background) samples.

3. "The sponsor provided hand-drawn diagrams and photocopies of ——— for each samples and quantiation of individual adduct spots within each sample."

Evaluation: As noted above, the sponsor in preliminary studies obtained a positive response with pantoprazole and subsequently reanalyzed samples using solvent system 1 without DNA adduct enhancement. ——— from these studies were impossible to interpret due to either ——— or difficulties in clearly photocopying ———. Referring to hand-drawn diagrams, resolution of adducts appeared to be poor and it is difficult to understand how the sponsor was able to subdivide large spots (i.e., masses) into individual adduct spots. Further, consistency in the subdivision of large spots into individual adduct spots was not apparent. It could not be discerned if the sponsor was using a constant exposure time for quantitation of adducts as 3 or 4 ——— varying in ——— were presented for each sample. Representative hand-drawn diagrams of ——— obtained with pantoprazole and control samples are shown below. Clearly, this data as presented is impossible to interpret and adds little assistance in interpretation of experiments. It should be noted that spots identified by the sponsor as omeprazole- or pantoprazole-specific adducts were separate and distinct.